Pathophysiology and Treatment of Hyperhomocysteinemia in End-Stage Renal Diseas Patients

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The pathophysiology of hyperhomocysteinemia in end-stage renal disease (ESRD) patients includes impaired remethylatio homocysteine (Hcy) to methionine, inhibition of extrarenal Hcy metabolism by uremic solutes, a block in decarboxylatio cysteinesulfinic acid, impaired [adenosylmethionine]/[adenosylhomocysteine] ratio, and a probable impairment of renal metabolism and excretion.

Treatment of hyperhomocysteinemia in ESRD patients includes administration of folic acid (1 – 15 mg per day). No additi effects have been observed with higher folic acid doses, folinic acid, or 5-methyltetrahydrofolate. Oral supplementation vitamin B6 and vitamin B12 has no effect, but some studies reported a decrease of plasma Hcy with high intravenous vita doses. Effective reduction of plasma total Hcy (tHcy) in patients treated with super-flux hemodialyzers suggests the remov uremic toxins with inhibitory activities against enzymes involved in the extrarenal Hcy metabolism.

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Key words

Homocysteine, renal failure, folic acid

Introduction

Moderate hyperhomocysteinemia (15 – 30 µmol/L) occurs in most patients with chronic renal failure [1]. Hyperhomocysteinemia adverse cardiovascular risk factor in end-stage renal disease patients [2–4]. Plasma total homocysteine (tHcy) consist approximately 70% protein-bound homocysteine (Hcy), bound via disulfide bonds mainly to plasma albumin [5], and about 25 30% free mixed disulfides, mostly homocysteine–cysteine [6,7], in the circulation. Approximately 2% of circulating tHcy is in reduced form (rHcy). Recently, Hoffer *at al.* [8] developed a sensitive method for measuring plasma rHcy concentrations. They f an average plasma tHcy concentration of 8.47 ± 0.58 µmol/L in normal adults whose rHcy concentration was 0.24 ± 0.03 µmol/L. pre-dialysis tHcy concentration in end-stage renal disease patients was 21.5 ± 1.1 µmol/L. Their pre-dialysis rHcy level was 0.

0.04 μmol/L. Hemodialysis therapy significantly lowered both tHcy and rHcy concentrations [8].

Homocysteine metabolism

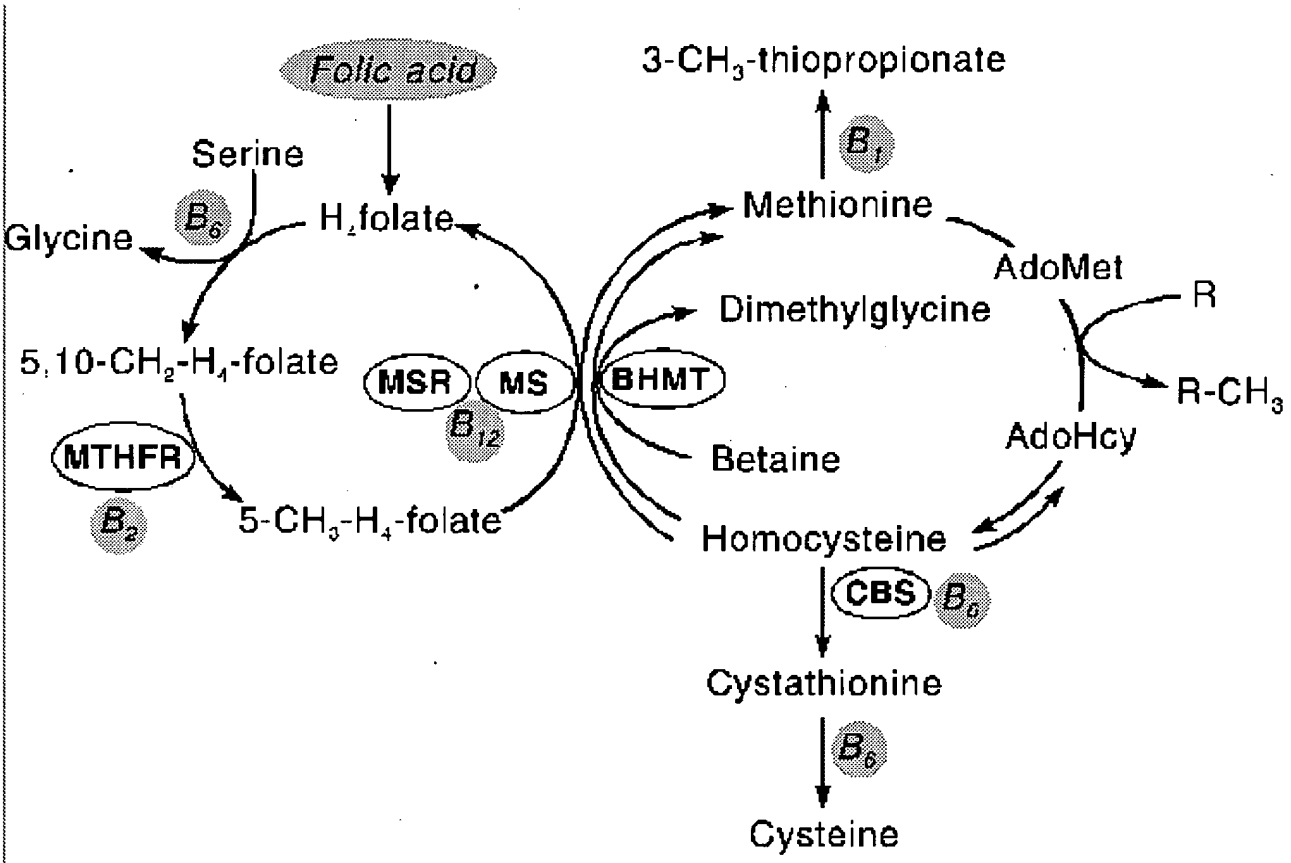

Fig. 1

shows a diagram of Hcy metabolism. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the folate cycle. In the 5-methyltetrahydrofolate (5-CH3-H4-folate) serves as a methyl donor and as a source of tetrahydrofolate (H4folate), synthesize methionine synthase reductase (MSR) and methionine synthase (MS). One of the reactions requiring 5,10-methylenetetrahydrof and 5-methyltetrahydrofolate is the synthesis of methionine from homocysteine, a remethylation pathway of the homocys metabolism. The second remethylation pathway involves **betaine**–homocysteine methyltransferase (BHMT) to form dimethylgly from **betaine**.

Homocysteine in the cell is derived from methionine. This reaction involves many transmethylation reactions (R: methyl g acceptor), producing *S*-adenosylhomocysteine (AdoHcy) from *S*-adenosylmethionine (AdoMet). Cystathionine β-synthase (C catalyzes the trans-sulfuration of homocysteine to cystathionine and cysteine.

Several factors contribute to the hyperhomocysteinemia of end-stage renal disease patients. Because urinary excretion of homocys is negligible in healthy subjects [5], a lack of elimination due to impaired renal function is improbable. On the other hand, eve renal failure patients, the kidney may contribute to homocysteine metabolism. Impaired non-renal disposal owing to inhibitio crucial enzymes in the methionine–homocysteine metabolism by the uremic milieu has been suggested [9]. High-dose folic a however, reduces plasma tHcy concentration by improving tissue Hcy remethylation [10].

Van Tellingen *et al.* [11] prospectively assessed tHcy levels in patients undergoing regular hemodialysis with either high polysulfone dialyzers (F60: Fresenius Medical Care, Bad Homburg, Germany) or super-flux dialyzers [polysulfone (F500S: Fresen and cellulosic triacetate (Tricea 150 G: Baxter Healthcare, Osaka, Japan)]. Total Hcy levels remained stable during high-flux dia therapy. However, tHcy decreased significantly to 21.5 ± 8.5 μmol/L (week 12) from 29.6 ± 9.9 μmol/L (week 1) during hemodia with F500S, and to 15.3 ± 3.7 μmol/L (week 12) from 24.4 ± 8.7 μmol/L (week 1) during hemodialysis with Tricea 150 G. Bec the molecular weight of free homocysteine is less than 268 Da (and not responsible for the observed reduction during super dialysis), the authors concluded that the most likely explanation seems to be the removal of uremic toxins with inhibitory activ against enzymes involved in the extrarenal homocysteine metabolism [11]. This mechanism has also been suggested by other aut [12,13] as an alternative explanation for the elevated tHcy levels in end-stage renal disease patients. However, the reduction of pla tHcy in patients treated with super-flux hemodialyzers may be the result of albumin loss, given that Hcy is 70% protein-bound.

noteworthy that the reduction in tHcy during hemodialysis is not markedly different between high-flux and low-flux devices

An elevation in plasma homocysteine concentration leads to an increased intracellular level of its precursor, adenosylhomocys [15], a potent inhibitor of all adenosylmethionine-dependent transmethylation reactions [16,17]. Consequently, [adenosylmethionine]/[adenosylhomocysteine] ratio has been used as a key metabolic parameter to evaluate the degree of inhibition [15,18].

Treatment of hyperhomocysteinemia with folates

Perna *et al.* [19] studied the metabolic effects of oral methyltetrahydrofolate, the active form of folic **acid**, on regular hemodia patients. Two months of therapy led to a significant reduction of plasma tHcy and to a significant amelioration of the ratio betw adenosylmethionine and adenosylhomocysteine. The data, however, could not be confirmed in another study. Bostom *et al.* treated two groups of 25 hemodialysis patients for 12 weeks with oral folic **acid** (15 mg daily) or an equimolar amount (17 mg d of oral l-5-methyltetrahydrofolate. All 50 subjects also received oral **vitamin** B6 (50 mg daily) and **vitamin** B12 (1 mg daily). mean percentage reductions were comparable between the two groups (14.8% vs 17.0%). Table I summarizes these studies and o homocysteine-lowering trials [21–46].

TABLE I Therapy of hyperhomocysteinemia in dialysis patients.

Author	*Study type*	*Substance and dosis*	*Main effect on tHcy*
Wilcken *et al.*, 1981 [21][a]	US	5 mg FA/PO/d + 100 mg B_6/PO/d + 1 mg B_{12}/IM	35% reduction of cysteine–homocysteine
Wilcken *et al.*, 1988 [22][a]	US	5 mg FA/PO/d	49% reduction of total fHcy
Arnadottir *et al.*, 1993 [23]	US	300 mg B_6/PO/d, then 5 mg FA/PO/d	B_6 no effect, 30% reduction by FA
Bostom *et al.*, 1995 [24]	US	6 g betaine/PO/d	No effect
Bostom *et al.*, 1995 [25]	US	3–4 g serine/PO/d	No effect
Bostom *et al.*, 1996 [26]	US	1200 mg N-acetylcysteine pre-HD	16% reduction
Bostom *et al.*, 1996 [27]	RCT	15 mg FA/PO/d + 100 mg B_6/PO/d + 1 mg B_{12}/PO/d vs. low dose vitamins	30% reduction by high dose vitamins
Perna *et al.*, 1997 [19]	US	15 mg MTHF/PO/d	72% reduction
van Guldener *et al.*, 1998 [28]	RCT	5 mg FA/PO/d vs 5 mg FA + 4 g betaine/PO/d	No additional effect of betaine
	RCT	then 5 mg FA/PO/d vs 1 mg FA/PO/d	5 mg FA not better than 1 mg FA
	US	then + 15 mg FA/PO/d	No additional effect
van Guldener *et al.*, 1998 [29]	RCT	5 mg FA/PO/d vs 5 mg FA + 4 g betaine/PO/d	No additional effect of betaine
	RCT	then 5 mg FA vs 1 mg FA/PO/d	5 mg FA not better than 1 mg FA
van Guldener *et al.*, 1999 [30]	RCT	5 mg FA/PO/d vs 4 g betaine/PO/d	Betaine not better than FA (F + PML)
	RCT	then 1 mg FA/PO/d vs 5 mg FA/PO/d	5 mg FA not better than 1 mg FA (F + PML
Dierkes *et al.*, 1999 [31]	RCT	2.5 mg FA/PO/HD vs 5 mg FA/PO/HD	5 mg FA not better than 2.5 mg FA
Dierkes *et al.*, 1999 [32]	US	1 mg B_{12}/IV/w	35% reduction (B_{12}-deficient patients)
Spence *et al.*, 1999 [33]	US	1 mg FA/PO/d vs 5 mg FA/PO/d	5 mg FA not better than 1 mg FA
Kunz *et al.*, 1999 [34]	RCT	10 mg FA/PO/d vs placebo	36.6% reduction
Suliman *et al.*, 1999 [35]	US	200 mg B_6/PO/d + 15 mg FA/PO/d	Decrease of fHcy
Touam *et al.*, 1999 [36]	US	50 mg FTHF/IV/w + 250 mg B_6/IV/HD	78% normalization (retrospective study)
Sunder-Plassmann *et al.*, 2000 [37]	RCT	15 mg vs 30 mg vs 60 mg FA/PO/d	30 mg and 60 mg FA not better than 15 mg F
Bostom *et al.*, 2000 [38]	RCT	15 mg FA/PO/d vs 17 mg MTHF/PO/d	MTHF not better than FA
Arnadottir *et al.*, 2000 [39]	US	15 mg, then 35 mg, then 70 mg FA/PO/w	35 or 70 mg FA not better than 15 mg FA
Tremblay *et al.*, 2000 [40]	RCT	1 mg FA vs 1 mg FA/PO/d + 10 mg FA/IV/HD	10 mg FA IV no additional effect
Thambyrajah *et al.*, 2000 [41][b]	RCT	5 mg FA/PO/d vs placebo	25% reduction vs placebo
Manns *et al.*, 2001 [42]	US	DiaVite™[c], addition of 1 mg B_{12}/PO/d	16.7% reduction
	RCT	Addition of placebo vs 5 mg vs 20 mg FA/PO/d	5 mg and 20 mg FA not better than 1 mg FA
	US	addition of 8 g serine/PO/d	No effect
Hauser *et al.*, 2001 [43]	RCT	15 mg FA/IV/HD vs 16.1 mg FTHF/IV/HD	FTHF not better than FA
Suliman *et al.*, 2001 [44]	US	200 mg B_6 + 15 mg FA/PO/d	Decrease of PML tHcy
Yango *et al.*, 2001 [45]	RCT	15 mg FA/PO/d vs 20 mg FTHF/PO/d	FTHF not better than FA
Dierkes *et al.*, 2001 [46]	RCT	1.6 mg FA + 12 μg B_{12} + 20 mg B_6/PO/HD vs 0.32 mg FA + 20 mg B_6/PO/HD vs placebo	1.6 mg FA + 12 μg B_{12} vs placebo better than 0.32 mg FA vs placebo

[a] First folic acid treatment studies in renal failure conducted with kidney graft recipients and chronic renal failure patients.
[b] One of the largest studies conducted in chronic renal failure patients.
[c] R&D Laboratories, Marina del Rey, CA, U.S.A.
tHcy = total homocysteine; US = uncontrolled study or case report; FA = folic acid; PO = orally; d = day; IM = intramuscularly; fHcy = free homocystein HD = hemodialysis session; RCT = randomized controlled trial; MTHF = 5-methyltetrahydrofolate; F = fasting; PML = post methionine loading; IV = intrav nously; w = week; FTHF = 5-formyltetrahydrofolate (folinic acid).

Suliman *et al.* [35] investigated the effects of supplementation with high doses of folic acid (15 mg daily) and pyridoxine (200 daily) on sulfur amino acid metabolism in red blood cells and plasma of regular hemodialysis patients and healthy subjects. therapy reduced the plasma tHcy concentration in both groups. In addition, plasma concentrations of cysteinylglycine, glutathione free cysteinesulfinic acid were significantly higher in hemodialysis patients as compared with healthy subjects. The authors sugge that a block in decarboxylation of free cysteinesulfinic acid is linked to hyperhomocysteinemia in ESRD patients

The hyperhomocysteinemia of patients with chronic renal insufficiency can usually be normalized with folic acid doses of 2 – 5 daily [47–49]. In contrast, end-stage renal disease patients are, to varying degrees, refractory to available treatments, including fol vitamin B6, and vitamin B12, even at high doses. It has been shown that, in nearly all hemodialysis patients, folic acid doses as as 60 mg daily fail to normalize plasma tHcy levels [37]. In a study by Tremblay *et al.* [40], the tHcy lowering effect of 10 mg of acid thrice weekly intravenously (IV) was not superior to 1 mg of folic acid orally per day.

Touam *et al.* [36] determined plasma tHcy concentrations before and during IV supplementation of folinic acid (50 mg once wee together with IV supplementation of pyridoxine (250 mg three times weekly). On folinic acid treatment, mean plasma tHcy le decreased significantly to 12.3 ± 5.4 μmol/L from 37.3 ± 5.8 μmol/L at baseline. At the end of follow-up, 29 of the 37 patients (7 investigated had normal plasma tHcy levels. No adverse effects attributable to folinic acid treatment were obser

Other investigators could not confirm these data, however. In a study by Yango *et al.* [45], two groups of 24 hemodialysis pat were treated for 12 weeks with oral folic acid (15 mg daily) or an equimolar amount (20 mg daily) of oral l-folinic acid. All pat also received oral vitamin B6 (50 mg daily) and vitamin B12 (1 mg daily). The mean percentage reductions in pre-dialysis tHcy comparable between hemodialysis patients on l-folinic acid [22.1% (range: 11.8% – 31.4%)] and patients on folic acid [20 (11.7% – 30.5%)]. The investigators concluded that, relative to high-dose folic acid, high-dose oral l-folinic acid –b supplementation does not improve tHcy-lowering efficacy in hemodialysis patients [45].

In a study by Hauser *et al.* [43], 66 hemodialysis patients were allocated to two groups of 33 patients each, one group receiving 15 of folic and the other, an equimolar amount (16.1 mg) of folinic acid. The folic and folinic acid was given IV at the end of hemodialysis session, three times per week for 4 weeks. The tHcy levels in all participating subjects decreased to an average of ± 7.9 μmol/L at week 4 from an average of 31.4 ± 24.9 μmol/L at baseline. Normalization of tHcy plasma levels after 4 week treatment was achieved in 16 patients (24.2%). No significant difference was seen in the efficacy of the two substances to lo elevated tHcy levels in hemodialysis patients.

Betaine supplementation may lower tHcy plasma levels in healthy individuals, but the tHcy-lowering effect seems smaller than established by folic acid therapy [50]. Mutations of betaine–homocysteine methyltransferase do not influence tHcy plasma le [51].

Hyperhomocysteinemia has been shown to be associated with impaired endothelium-dependent NO-mediated vasodilation [52– This endothelial dysfunction in hyperhomocysteinemic subjects with [55] or without [56,57] clinically manifest vascular disorde improved, in patients with normal kidney function, by the administration of folates. Supplementation with folic acid also impr endothelial dysfunction in patients with familial hypercholesterolemia and normal tHcy [58]. In contrast, folates failed to signific improve endothelial dysfunction in patients undergoing regular hemodialysis treatment [28], in patients on peritoneal dialysis [29] in patients with pre-dialysis renal failure [41]. High-dose IV folic acid or folinic acid also failed to improve mean arterial pressu pulse pressure in hemodialysis patients [59].

Massy *et al.* [60] found a significant correlation between moderate hyperhomocysteinemia and plasmatic activity of glutath peroxidase in a large cohort of uremic patients. Folinic acid supplementation has also been shown not only to decrease tHcy but to prevent lipid peroxidation in hemodialysis patients [61]. These data suggest that moderate hyperhomocysteinemia may predis to endothelium dysfunction through a mechanism that involves the generation of reactive oxygen species

Oxidative stress may be involved in abnormalities of coagulation associated with hyperhomocysteinemia. Reactive oxygen spe generated during Hcy auto-oxidation, initiate lipid peroxidation in cell membranes and circulating lipoproteins, resulting in pla activation and hemostatic abnormalities [62]. Markedly elevated Hcy levels in patients with inborn errors of Hcy metabolism resu thromboembolic disease [63].

Conclusion

The pathophysiology of hyperhomocysteinemia in ESRD patients is not entirely understood. Most of these patients are refractory t usual therapies. Hopefully, this important issue will be resolved.

References

1. Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: Prevalence, etiology, and potential relationship of arteriosclerotic outcomes.

Kidney Int. 52(1): 10–20, 1997.
2. Bostom AG, Shemin D, Lapane KL, Sutherland P, Nadeau MR, Wilson PW, Yoburn D, Bausserman L, Tofler G, Jacques PF, Selhub J, Rosenberg IH. Hyperhomocysteinemia, hyperfibrinogenemia, and lipoprotein(a) excess in maintenance dialysis patients: A matched case-control study. Atherosclerosis. 125(1):91–101, 1996.
3. Robinson K, Gupta A, Dennis V, Arheart K, Chaudhary D, Green R, Vigo P, Mayer EL, Selhub J, Kutner M, Jacobsen DW. Hyperhomocysteinemia con an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. Circulatio 94(11):2743–8, 1996.
4. Moustapha A, Naso G, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, Robinson K, Dennis VW. Prospective study of hyperhomocysteinemia as an ad cardiovascular risk factor in end-stage renal disease. Circulation. 97(2):138–41, 1998.
5. Refsum H, Helland S, Ueland PM. Radioenzymic determination of homocysteine in plasma and urine. Clin Chem. 31(4):624–8, 1985.
6. Gupta VJ, Wilcken DE. The detection of cysteine–homocysteine mixed disulphide in plasma of normal fasting man. Eur J Clin Invest. 8(4):205–7, 1978
7. Ueland PM. Homocysteine species as components of plasma redox thiol status. Clin Chem. 41(3):340–2, 1995.
8. Hoffer LJ, Robitaille L, Ellan KM, Bank I, Hongsprabhas P, Mamer OA. Plasma reduced homocysteine concentrations are increased in end-stage renal disease. Kidney Int. 59(1): 372–7, 2001.
9. van Guldener C, Robinson K. Homocysteine and renal disease. Semin Thromb Hemost. 26(3):313–24, 2000.
10. Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. Kidney Int. 52(2):495–502, 1997.
11. Van Tellingen A, Grooteman MPC, Bartels PCM, van Limbeek J, van Guldener J, ter Wee PM, Nubé MJ. Long-term reduction of plasma homocysteine levels by super-flux dialyzers in hemodialysis patients. Kidney Int. 59(1):342–7, 2001.
12. van Guldener C, Donker AJM, Jacobs C, Teerlink T, de Meer K, Stehouwer CD. No net renal extraction of homocysteine in fasting humans. Kidney Int. 54(1):166–9, 1998.
13. Arnadottir M, Berg AL, Hegbrant J, Hultberg B. Influence of hemodialysis in plasma total homocysteine concentration. Nephrol Dial Transplant. 14(1):142–6, 1999.
14. Biasioli S, Schiavon R, Petrosino L, Cavallini L, Cavalcanti G, De Fanti E. Dialysis kinetics of homocysteine and reactive oxygen species. ASAIO J. 44(5):423–32, 1998.
15. Perna AF, Ingrosso D, Zappia V, Galletti P, Capasso G, De Santo NG. Enzymatic methyl esterification of erythrocyte membrane proteins is impaired in chronic renal failure: Evidence for high levels of the natural inhibitor *S*-adenosyl-homocysteine. J Clin Invest. 91(6):2497–503, 1993.
16. Zappia V, Zydek–Cwick CR, Schlenk F. The specificity of *S*-adenosylmethionine derivatives in methyl transfer reactions. J Biol Chem. 244(16):4499–5 1969.
17. Barber JR, Clarke S. Inhibition of protein carboxyl methylation by *S*-adenosyl-l-homocysteine in intact erythrocytes: Physiological consequences. J Biol Chem. 259(11):7115–22, 1984.
18. Perna AF, Ingrosso D, Galletti P, Zappia V, De Santo NG. Membrane protein damage and methylation reactions in chronic renal failure. Kidney Int. 50(2):358–66, 1996.
19. Perna AF, Ingrosso D, De Santo NG, Galletti P, Brunone M, Zappia V. Metabolic consequences of folate-induced reduction of hyperhomocysteinemia in uremia. J Am Soc Nephrol. 8(12):1899–905, 1997.
20. Bostom AG, Shemin D, Gohh RY, Beaulieu AJ, Bagley P, Massy ZA, Jacques PF, Dworkin L, Selhub J. Treatment of hyperhomocysteinemia in hemodialysis patients and renal transplant recipients. Kidney Int. 59(suppl 78):S246–52, 2001.
21. Wilcken DE, Gupta VJ, Betts AK. Homocysteine in the plasma of renal transplant recipients: Effects of cofactors for methionine metabolism. Clin Sci. 61(6):743–9, 1981.
22. Wilcken DE, Dudman NP, Tyrrell PA, Robertson MR. Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: Possible implicati for prevention of vascular disease. Metabolism. 37(7):697–701, 1988.
23. Arnadottir M, Brattström L, Simonsen O, Thysell H, Hultberg B, Andersson A, Nilsson–Ehle P. The effect of high-dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. Clin Nephrol. 40(4):236–40, 1993.
24. Bostom AG, Shemin D, Nadeau MR, Shih V, Stabler SP, Allen RH, Selhub J. Short term betaine therapy fails to lower elevated fasting total plasma homocysteine concentrations in hemodialysis patients maintained on chronic folic acid supplementation (letter). Atherosclerosis. 113(1): 129–32, 1995.
25. Bostom AG, Shemin D, Lapane KL, Miller JW, Sutherland P, Nadeau M, Seyoum E, Hartman W, Prior R, Wilson PWF, Selhub J. Hyperhomocysteinem and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: A case-control study. Atherosclerosis. 114(1): 93–103, 1
26. Bostom AG, Shemin D, Yoburn D, Fisher DH, Nadeau MR, Selhub J. Lack of effect of oral n-acetylcysteine on the acute dialysis-related lowering of tot plasma homocysteine in hemodialysis patients. Atherosclerosis. 120(2):241–4, 1996.
27. Bostom AG, Shemin D, Lapane KL, Hume AL, Yoburn D, Nadeau MR, Bendich A, Selhub J, Rosenberg IH. High dose–B-vitamin treatment of hyperhomocysteinemia in dialysis patients. Kidney Int. 49(1):147–52, 1996.
28. van Guldener C, Janssen MJFM, Lambert J, ter Wee PM, Jakobs C, Donker AJ, Stehouwer CD. No change in impaired endothelial function after long-te folic acid therapy of hyperhomocysteinaemia in haemodialysis patients. Nephrol Dial Transplant. 13(1):106–12, 1998.
29. van Guldener C, Janssen MJFM, Lambert J, ter Wee PM, Donker AJM, Stehouwer CDA. Folic acid treatment of hyperhomocysteinemia in peritoneal di patients: No change in endothelial function after long-term therapy. Perit Dial Int. 18(3):282–9, 1998.
30. van Guldener C, Janssen MJ, de Meer K, Donker AJ, Stehouwer CD. Effect of folic acid and betaine on fasting and postmethionine-loading plasma homocysteine and methionine levels in chronic haemodialysis patients. J Intern Med. 245(2):175–83, 1999.
31. Dierkes J, Domröse U, Ambrosch A, Bosselmann HP, Neumann KH, Luley C. Response of hyperhomocysteinemia to folic acid supplementation in patie with end-stage renal disease. Clin Nephrol. 51(2):108–15, 1999.
32. Dierkes J, Domröse U, Ambrosch A, Schneede J, Guttormsen AB, Neumann KH, Luley C. Supplementation with vitamin B12 decreases homocysteine methylmalonic acid but also serum folate in patients with end-stage renal disease. Metabolism. 48(5):631–5, 1999.
33. Spence JD, Cordy P, Kortas C, Freeman D. Effect of usual doses of folate supplementation on elevated plasma homocyst(e)ine in hemodialysis patients: N difference between 1 and 5 mg daily. Am J Nephrol. 19(3):405–10, 1999.
34. Kunz K, Petitjean P, Lisri M, Chantrel F, Koehl C, Wiesel ML, Cazenave JP, Moulin B, Hannedouche TP. Cardiovascular morbidity and endothelial dysfunction in chronic haemodialysis patients: Is homocyst(e)ine the missing link? Nephrol Dial Transplant. 14(8):1934–42, 1999.
35. Suliman ME, Divino Filho JC, Bárány P, Anderstam B, Lindholm B, Bergström J. Effects of high-dose folic acid and pyridoxine on plasma and erythroc sulfur amino acids in hemodialysis patients. J Am Soc Nephrol. 10(6): 1287–96, 1999.
36. Touam M, Zingraff J, Jungers P, Chadefaux–Vekemans B, Drüeke T, Massy ZA. Effective correction of hyperhomocyst(e)inemia in hemodialysis patien intravenous folinic acid and pyridoxine therapy. Kidney Int. 56(6):2292–6, 1999.
37. Sunder–Plassmann G, Födinger M, Buchmayer H, Papagiannopoulos M, Wojcik J, Kletzmayr J, Enzenberger B, Janata O, Winkelmayer WC, Paul G, Auinger M, Barnas U, Hörl WH. Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: Results of the Vienna Multice Study. J Am Soc Nephrol. 11(6):1106–16, 2000.
38. Bostom AG, Shemin D, Bagley P, Massy ZA, Zanabli A, Christopher K, Spiegel P, Jacques PF, Dworkin L, Selhub J. Controlled comparison of l-5-methyltetrahydrofolate versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. Circulation. 101(24):2829–32, 2000.
39. Arnadottir M, Gudnason V, Hultberg B. Treatment with different doses of folic acid in haemodialysis patients: Effects on folate distribution and aminoth concentrations. Nephrol Dial Transplant. 15(4):524–8, 2000.

40. Tremblay R, Bonnardeaux A, Geadah D, Busque L, Lebrun M, Ouimet D, Leblanc M. Hyperhomocysteinemia in hemodialysis patients: Effects of 12–m supplementation with hydrosoluble vitamins. Kidney Int. 58(2):851–8, 2000.
41. Thambyrajah J, Landray MJ, McGlynn FJ, Jones HJ, Wheeler DC, Townend JN. Does folic acid decrease plasma homocysteine and improve endothelia function in patients with pre-dialysis renal failure? Circulation. 102(8):871–5, 2000.
42. Manns B, Hyndman E, Burgess E, Parsons H, Schaefer J, Snyder F, Scott–Douglas N. Oral vitamin B12 and high-dose folic acid in hemodialysis patien with hyper-homocyst(e)inemia. Kidney Int. 59(3):1103–9, 2001.
43. Hauser AC, Hagen W, Rehak PH, Buchmayer H, Födinger M, Papagiannopoulos M, Bieglmayer C, Apsner R, Köller E, Ignatescu M, Hörl WH, Sunder–Plassmann G. Efficacy of folinic versus folic acid for the correction of hyperhomocysteinemia in hemodialysis patients. Am J Kidney Dis. 37(4) 758–65, 2001.
44. Suliman ME, Filho JC, Bárány P, Anderstam B, Lindholm B, Bergström J. Effects of methionine loading on plasma and erythrocyte sulphur amino acids sulph-hydryls before and after co-factor supplementation in haemodialysis patients. Nephrol Dial Transplant. 16(1):102–10, 2001.
45. Yango A, Shemin D, Hsu N, Jacques PF, Dworkin L, Selhub J, Bostom AG. l-Folinic acid versus folic acid for the treatment of hyperhomocysteinemia hemodialysis patients. Kidney Int. 59(1):324–7, 2001.
46. Dierkes J, Domröse U, Bosselmann KP, Neumann KH, Luley C. Homocysteine lowering effect of different multivitamin preparations in patients with end-stage renal disease. J Ren Nutr. 11(2):67–72, 2001.
47. Beaulieu AJ, Gohh RY, Han H, Hakas D, Jacques PF, Selhub J, Bostom AG. Enhanced reduction of fasting total homocysteine levels with supraphysiolo versus standard multivitamin dose folic acid supplementation in renal transplant recipients. Arterioscler Thromb Vasc Biol. 19(12): 2918–21, 1999.
48. Bostom AG, Gohh RY, Beaulieu AJ, Nadeau MR, Hume AL, Jacques PF, Selhub J, Rosenberg IH. Treatment of hyperhomocysteinemia in renal transpl recipients: A randomized, placebo-controlled trial. Ann Intern Med. 127(12):1089–92, 1997.
49. Jungers P, Joly D, Massy Z, Chauveau P, Nguyen AT, Aupetit J, Chadefaux B. Sustained reduction of hyperhomocysteinemia with folic acid supplementation in pre-dialysis patients. Nephrol Dial Transplant. 14(12):2903–6, 1999.
50. Heil SG, Lievers KJ, Boers GH, Verhoef P, den Heijer M, Trijbels FJ, Blom HJ. Betaine–homocysteine methyltransferase (BHMT): Genomic sequencin and relevance to hyperhomocysteinemia and vascular disease in humans. Mol Genet Metab. 71(3):511–19, 2000.
51. Brouwer IA, Verhoef P, Urgert R. Betaine supplementation and plasma homocysteine in healthy volunteers. Arch Intern Med. 160(16):2546–7, 2000.
52. Tawakol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. Circulation. 95(5):1119–21, 1997.
53. Chambers JC, McGregor A, Jean–Marie J, Kooner JS. Acute hyperhomocysteinaemia and endothelial dysfunction. Lancet. 351(9095):36–7, 1998.
54. Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Bones C, Newcombe RG, Lewis MJ. Hyperhomocysteinemia after an oral methionine load acut impairs endothelial function in healthy adults. Circulation. 98(18):1848–52, 1998.
55. Chambers JC, Ueland PM, Obeid OA, Wirgley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins: An effect mediate through reduced concentrations of free plasma homocysteine. Circulation. 102(20): 2479–83, 2000.
56. Bellamy MF, McDowell IF, Ramsey MW, Brownlee M, Newcombe RG, Lewis MJ. Oral folate enhances endothelial function in hyperhomocysteinaemic subjects. Eur J Clin Invest. 29(8):659–62, 1999.
57. Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C, Celermajer DS. Folic acid improves arterial endothelial function in adults with hyperhomocysteinemia. J Am Coll Cardiol. 34(37):2002–6, 1999.
58. Verhaar MC, Wever RM, Kastelin JJ, van Loon D, Milstien S, Koomans HA, Rabelink TJ. Effects of oral folic acid supplementation on endothelial func in familial hypercholesterolemia. A randomized placebo-controlled trial. Circulation. 100(4):335–8, 1999.
59. Hagen W, Kletzmayr J, Födinger M, Hauser AC, Hörl WH, Sunder–Plassmann G. Effect of intravenous folic acid or folinic acid therapy on mean arteri pressure and pulse pressure in hemodialysis patients. Am J Hypertens. 14(4 suppl 1):A87–8, 2001.
60. Massy ZA, Ceballos I, Chadefaux–Vekemens B, Nguyen–Khoa T, Descamps–Latscha B, Drüeke TB, Jungers P. Homocyst(e)ine, oxidative stress, and endothelium function in uremic patients. Kidney Int. 59(suppl 78):S243–5, 2001.
61. Bayes B, Bonal J, Pastor C, Romero R. Lipid peroxidation in hemodialysis: Preventive role of vitamin E and folinic acid (abstract). J Am Soc Nephrol. 10:274A, 1999.
62. Coppola A, Davi G, De Stefano V, Mancini FP, Cerbone AM, Di Minno G. Homocysteine, coagulation, platelet function, and thrombosis. Semin Throm Hemost. 26(3): 243–54, 2000.
63. Langman LJ, Cole DE. Homocysteine. Crit Rev Clin Lab Sci. 36(4):365–406, 1999.